

## Shared Beringia Heritage Program

J9911090040 - Unraveling the Evolutionary Nature of Beringian Plants

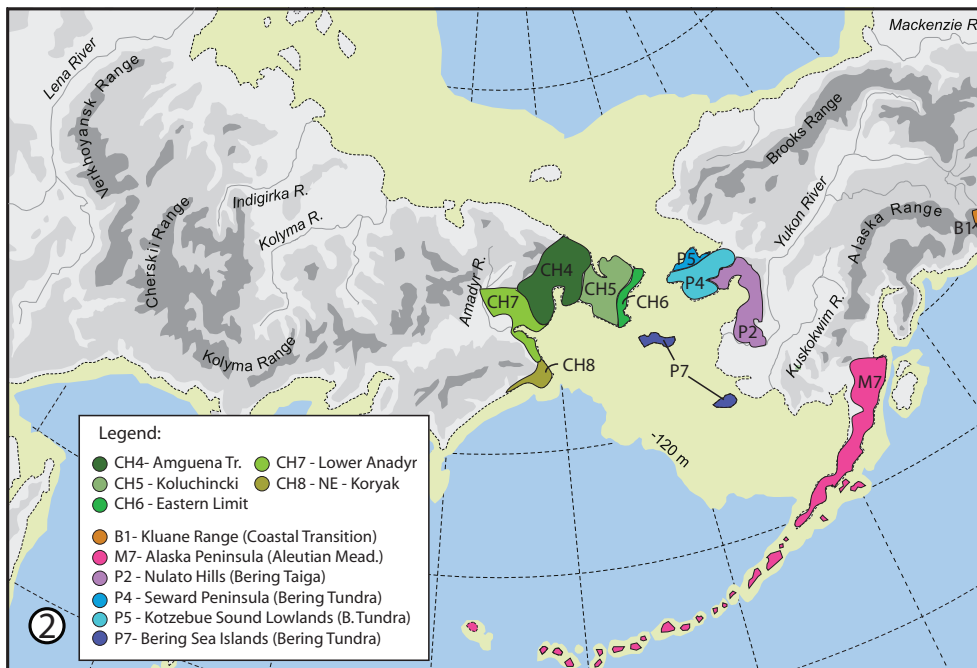
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### INTERIM REPORT AND ADDITIONAL FUNDING REQUEST

The field component of this project has been successfully completed in July 2010. We made 271 voucher specimens (254 in the Chukotka Region and 17 in the Provideniya Region) and took over 80 different population samples (10 samples per population with a single voucher per population). We collaborated with two researchers from the Komarov Botanical Institute, Dr. Nadja Sekretareva, and a Ph.D. student, Julia Mikhailova. Their field assistance was invaluable to the success of this project. We have interacted with the natural history museum in Provideniya and presented a powerpoint presentation of our project to them. Back in Alaska we have presented preliminary results at the Alaska Botany Forum, organized by the National Park Service (Ickert-Bond et al., 2011a). We contributed numerous field images to the Shared Beringia Heritage Program 2011 calendar. Ickert-Bond also presented her preliminary findings in the form of a poster presentation at the Beringia Days Conference in Nome in Sept. 2011 (Ickert-Bond et al. 2011b).

### PRELIMINARY RESULTS

We carried out a detailed collecting program of Beringian plants to the Chegitun River, eastern Chukotka (Western Beringia) in the summer of 2010 in collaboration with the Komarov Botanical Institute, St. Petersburg to compare with taxa from collecting efforts on the Seward Peninsula in Alaska (Eastern Beringia).



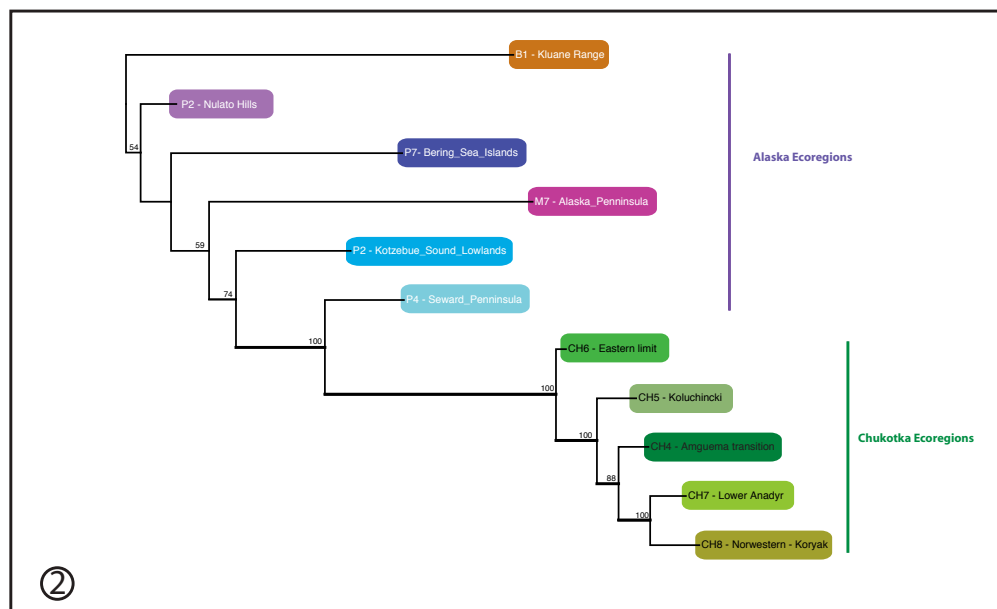
**Fig. 1** – Mapping of selected Alaska ecoregions (after Nowicki et al. 2001) and those from Chukotka (Yurtsev et al. 2010) used in this study with the maximum extent of the Bering Land Bridge during the Last Glaciation shown in yellow.

**Materials and Methods:**

- 1) Extract georeferenced records (103,074) from UA Museum herbarium database <http://arctos.database.museum/SpecimenSearch.cfm> for ecoregions in Alaska (Nowicki et al. 2001) as well as from the checklist of *Flora of the Chukotkan Tundra* (Yurtsev et al. 2010). Taxonomy following the Panarctic Flora checklist 2011 <http://www.nhm.uio.no/english/research/infrastructure/paf/>
- 2) Construct presence-absence taxon matrix for all ecoregions (Fig. 1).
- 3) Examine the dataset in a Maximum Likelihood framework using a simple Markov k model and Gamma distribution rates of heterogeneity in RAxML (Cipres Portal).
- 4) Using the resulting taxon-area cladogram as the basis for stochastic key species reconstruction in the program Mesquite <http://mesquiteproject.org/mesquite/> we can generate testable hypotheses about the history of plant populations in Beringia.

### Results:

The matrix contained 2325 unique taxon names. Many are shared across both Alaska and Chukotka. The richest communities are the Seward Peninsula in AK and the *Chukotkan 6 – Eastern Limit region* with 775 taxa and 597 taxa respectively (Table 1). The poorest are the *Bering Sea Islands* in AK with 275 taxa and the *CH4 – Amguema transition* and *CH5- Koluchinski* each with 470 taxa. Maximum likelihood (ML) analysis in RAxML unambiguously groups the Chukotkan ecoregions together (ML bootstrap support 100%) derived from within the AK ecoregions. The *Seward Peninsula ecoregion* shares the most taxa with Chukotka and groups unambiguously as the sister to the Chukotkan regions (ML BS 100%, Figure 3).



**Figure 2.** – Area cladogram of ecoregions based on ML analysis in RAxML vers. 7.28. Support values (ML BS) are indicated above branches based on 1000 replicates. Color of regions matches those in Figure 1.

**Conclusion:** The history of many plant taxa occurring in different areas/ ecosystems in Beringia cannot be explained by a single process of vicariance. Instead many populations may be the result of dispersal into the area. Overall,

dispersal is the most likely process for many endemic species distributions. In the absence of phylogenetic data, species occurrence records (from our collecting efforts in Chukotka and Alaska) can allow us to generate testable hypotheses about the history of populations in a given area that can be explored using genetic data.

## **ADDITIONAL GENETIC ANALYSES**

### **Complications:**

The fieldwork was extremely expensive and no more funds are remaining for the analytical component of the research project. In addition to the logistic costs we had to pay in Russia and mailing our specimens to St. Petersburg from Chukotka, we had to pay an additional \$1000 for preparing the specimens for shipment back to the USA (see attached invoice). We have made this payment, but our voucher specimens are still held up in St. Petersburg at the Komarov Botanical Institute. A Norwegian colleague was able to get the DNA samples out of Russia in March 2011. Recently, a Finnish visiting scholar at UAF told me he could possibly arrange for a Russian colleague, that now lives in Finland, to get the specimens back to Helsinki and then mail them to us at UAF. We are hopeful that the voucher specimens will arrive soon at UAF, as we will need these to verify identifications in the future.

With the DNA samples at hand we are ready to proceed with the detailed genetic analyses to produce a first pass at understanding genetic variation in Beringian plants. We were able to secure samples from Western Beringia (Chukotka) to compare with those from Eastern Beringia (Alaska). Recent new technological advances will allow us to produce large amount of data with the samples from Chukotka. Restriction-site associated DNA sequencing (RADSeq, Davey & Blaxter 2010), a method that samples at reduced complexity across target genomes, promises to deliver high resolution population genomic data - thousands of sequenced markers across many individuals - for any organism at reasonable costs. These next generation sequencing approaches are slightly more expensive than the traditional sequencing approaches we budgeted for in our original SBHP proposal in 2009. Getting data from these cutting-edge Next generation sequencing approaches will allow us to prepare a highly competitive proposal to the National Science Foundation for more expanding analysis and future collecting which is needed in interior Chukotka and SW Chukotka.

**Table 1. Cost for extractions kits, barcodes, library prep, restriction enzymes and sequencing for ca. 12 population samples (10-20 individuals per population).**

<b>Item</b>	<b>Request to SBHP</b>	<b>Ickert-Bond lab</b>
DNA extraction		\$1,495
barcodes (12 barcodes)	\$600	
Illumina lane	\$2,083	
restriction enzymes, PCR purification & gel extr. kits, ligase	\$500	
PCR primers	\$40	
<b>Total</b>	<b>\$3,223</b>	

Other consumables will be available in the Ickert-Bond lab.

**Methods:**

We will use Single nucleotide polymorphism (SNP) discovery and genotyping as essential genetic mapping tools. A simple, inexpensive platform that allows high-density SNP discovery and genotyping in large populations has recently been developed:

Sequencing of restriction-site associated DNA (RAD) tags (RADSeq), which can identify 10,000 SNPs, using Illumina sequencing technology (Davey & Blaxter 2010). We will target 5 populations (3 from Chukotka and 2 from Alaska) of the charismatic *Therorhodon camtschaticum* and *Therorhodon glandulosum* as well as an additional 7 populations of another Beringian taxon.

**References:**

- Davey J.M. and M.L. Blaxter . 2010. RADSeq: next-generation population genetics. Briefings in Functional Genomics 9(5): 416- 423.
- Ickert-Bond, S.M. and J. Metzgar. 2011a. Plant collecting expedition to the Chegitun River, eastern Chukotka, Russia in collaboration with the Komarov Botanical Institute, St. Petersburg. Alaska Botany Forum, NPS, Fairbanks, AK.
- Ickert-Bond, S.M., Metzgar, J.S., Jeffers, S., Sekretareva, N., & Y. Mikhaylova. 2011b. Unravelling evolutionary relationships of Beringian plants - Plant collecting expedition to the Chegitun River, eastern Chukotka in collaboration with the Komarov Botanical Institute, St. Petersburg. 2011 Beringia Days Conference, Shared Beringia Heritage Program, NPS, Nome, AK.